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THE EFFECT OF EXPOSURE TO COLD ON ANTIBODY PRODUCTION

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While it is a generally accepted belief that cold is an etiologic factor in the production of disease, especially of the respiratory tract, its effect on antibody production and leukocytic activity has not been studied thoroughly. Numerous experiments have been made to determine the anatomic and pathologic changes produced by cold without the intervention of infectious organisms, and much more experimental work has been done on the effect of cold on the susceptibility to bacterial and protozoon infections, but in most of the latter group of experiments no analyses of the antibody production have been made, mainly because of the fact that most of this work was done before immunology reached its present position. Brief mention of the results obtained by various observers may be made here, the summary of the literature including the results of chilling experiments for other purposes than to determine the influence of cold on the production of immune bodies.

In regard to the anatomic and pathologic changes produced by exposure to cold per se, the work of Walther,[1] in 1862, is perhaps the first. He showed that chilling rabbits until their rectal temperature had decreased 18-20 C. caused a suppression of their secretions, and, if the animals died, hyperemia and edema of the lungs and air passages and a hydrothorax similar to pleurisy with effusion were found. Lassar[2] demonstrated that chilling caused diarrhea in many cases and an albuminuria with hyaline casts 1 to 2 days after the chilling; microscopically the kidneys gave a constant picture of interstitial inflammation. Myer, Lierheim and Siegel[3] caused albuminuria and hematuria by immersing dogs in cold water. Sections of the kidneys showed cloudy swelling, hyaline and necrotic patches and hemorrhage. Preble[4] obtained similar results, using rabbits, and he also found that pleural and pericardial hemorrhages were frequent in animals chilled to death. Aufrecht[5] reports experiments on 3 rabbits in which he thinks he has shown that chilling causes thrombosis and hemorrhages in the lungs.

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[1] Virchows Arch., 1862, 25, p. 414.

[2] Ibid., 1880, 79, p. 168.

[3] Ztschr. f. Exp. Path. u. Ther., 1911, 9, p. 450.

[4] Ref. Handbook for Med. Sci., 154.

[5] Deut. Arch. f. klin. Med., 1915, 117, p. 602.

No other observers have corroborated the finding of coagulation of the blood in the vessels except Lassar, who found thrombi in the arteries of the viscera of chilled rabbits. Lang[6] did not find any coagulation from freezing localized areas, nor did Hochhaus[7] demonstrate thrombosis of the vessels in the kidney and liver to which he applied — 80° R. for 30 seconds.

Of the work done on establishing the rôle of cold as a factor in disease production and in the progress of disease already begun, Pasteur's experiments in 1878 on anthrax are the first to appear in the literature. These well known experiments were conducted as follows: Hens were inoculated with anthrax and half of them cooled in water, covering one third of their bodies, resulting in a fall of temperature from 42 to 38 C. All the chilled fowls developed the disease while the controls lived. Lock verified the results of Pasteur as did also Wagner,[8] who decreased the temperature by both antipyretics and the cold bath. Sawtschenko[9] obtained the same results with doves, and Dieudonne,[10] Petruchsky,[11] Nuttall,[12] and Metschnikoff[13] have shown that frogs are made susceptible to anthrax if they are warmed up and kept at 37 C.

Lode[14] in an extensive series of experiments found that guinea-pigs chilled in various ways were decidedly more susceptible to experimental infections with the pneumobacillus introduced either subcutaneously or by inhalation, with Staphylococcus aureus, cholera vibrio, B. anthracis and B. tuberculosis, than were controls similarly inoculated but not exposed to cold. Also, the mortality of animals already infected was shown to be increased greatly by exposure to cold. Lipari[15] and Rovighi[16] both found that chilled animals were much more susceptible to pneumococcus infections and the mortality was much higher than in control animals. Dürck[17] found that pneumonia developed without experimental infection in many of his animals which he had chilled in ice water, but never in his controls. Miller and Noble[18] found that chilled rabbits were decidedly more susceptible to B. bovisepticus (snuffles) than controls.

Ernst[19] injected frogs with B. ranicida and found that no infection resulted in the hot summer months, but in the cool spring or fall, or if the frogs were put in the ice-chest, they fell rapidly victims to the infection. Also he showed that if frogs were inoculated at a low temperature and a short time afterward heated up to summer temperature, no disease resulted. Filehne[20] found that after infecting rabbits' ears in the middle with erysipelas, the infection remained latent in the rabbits kept at 0 for 3 days, but severe and extensive erysipelas developed throughout the entire ear when the animal was transferred to room temperature, while in control rabbits the disease was much more moderate. He concludes that the bacteria had progressed to all parts of the ear by means of the lymph channels but that extensive growth was not allowed because of anemia from the cold. Ciuca[21] and Vincent[22] demonstrated that tetanus could be produced in white mice inoculated with tetanus spores, by immersing for a considerable time in a freezing mixture. With rabbits, exposure of the entire animal was not sufficient to induce tetanus in animals injected with spores, but exposure of an injected extremity almost invariably resulted in the development of the disease. Vincent explains this phenomenon on the ground that the temperature of the isolated extremity is easily reduced to a temperature at which the phagocytic activity is destroyed, whereas it is impossible to decrease the temperature of the whole body to such a degree.

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[6] Abstract in Jour. Am. Med. Assn., 1915, 64.

[7] Virchows Arch., 1898, 154, p. 320.

[8] Centralbl. f. Bacteriol., 1891, 9, p. 322.

[9] Ibid., 1891-1899, pp. 473, 493, and 528.

[10] Arbeiten aus Kaiser. Gesundheitsamt., 1894, 9, p. 492.

[11] Beit. path. Anat., 1888, 3, p. 359.

[12] Ztschr. f. Hyg., 1888, 4, p. 353.

[13] Virchows Arch., 1884, 97, p. 502.

[14] Arch. f. Hyg., 1897, 28, p. 344.

[15] Deut. med. Wchnschr., 1890, 16, p. 44.

[16] Virchow and Hirsch Jahres. gesamt. Med., 1892, 1.

[17] Deut. Arch. f. klin. Med., 1897, 58, p. 368.

[18] Jour. Exper. Med., 1916, 24, p. 223.

[19] Beitr. path. Anat., 1890, 8, p. 213.

[20] Jour. Physiol., 1894-95, 17, p. 315.

Ross and Williams[23] demonstrated that rats inoculated with trypanosomes (Evansi, Lewisi and Brucei) resisted the infection decidedly more than controls if they were kept in a cool chamber (20-36 F.). Ross and Thompson[24] showed the same thing for guinea-pigs inoculated with T. gambienzi.

Only two observers have failed to find a decreased resistance to bacterial infections when the host is exposed to cold. Pawlowsky[25] found that intense chilling—17 degrees for 15-30 minutes, failed to cause any decreased resistance to staphylococcus infections in rabbits, although many of his rabbits died from the cold directly. The other observer is Chodounsky,[26] who contests strenuously the belief that cold increases the susceptibility of laboratory animals or man to either artificial or autoinfection, especially referring to diseases of the respiratory tract. He cites his experiments on animals to the effect that pneumonia is not produced any easier in chilled than in control animals; he also presents results of experiments on himself to prove his point. He was 57 years old, subject to catarrh and frequent attacks of bronchitis, but nevertheless exposed himself after a hot bath, without drying, for periods of 20 minutes to an hour in draughts of cold air of 3-12 C. No pneumonia or serious results occurred.

Concerning the effect of cold on the cellular content of the blood, Rovighi, Winternitz,[27] and Schnützen[28] found that in both man and laboratory animals chilling causes a peripheral hyperleukocytosis, especially polymorphonuclear, which lasts for several hours, and Winternitz showed that there is considerable increase of the red cells (and increased hemoglobin index) in the peripheral blood. Rovighi and Secchi[29] showed that excessive cooling resulted in a decrease of the leukocytes, but an increase occurred when the heart action and respiration picked up again.

The conclusion to be drawn from the work on the effect of cold on phagocytosis, is that the leukocytes in chilled animals are less active than those of normal animals; and if leukocytes from normal animals are cooled in vitro their phagocytic activity is decreased. To this effect are the following experiments: Wagner demonstrated that the phagocytic activity of the leukocytes in hens injected with anthrax was reduced to a minimum in hens which had been cooled in a cold bath until the body temperature was reduced 2-3 C. Sanarelli[30] found that the leukocytes of chilled guinea-pigs were less phagocytic to Sp. metchnikovii than those of controls after the animals were chilled, while Lode, studying the bacterial content of the leukocytes around the point of injection in the experiments cited, found that the leukocytes of control animals did not contain any more bacteria than the leukocytes in chilled animals. Dean[31] found that the action of opsonin on staphylococci was nearly 10 times as great at 37 as at 6 C., and Rolly and Meltzer[32] found that the phagocytic power of human leukocytes for typical bacilli increased gradually from 6 to 37.5 C., but quite rapidly from 37.5 to 40 C. Trommsdorff[33] found that in guinea-pigs which he injected intraperitoneally with hemolyzed hen corpuscles there was decreased migration of leukocytes into the cavity and that there was also a decreased phagocytosis of the corpuscles in the cooled animals. Ledingham[34] showed that when leukocytes and staphylococci were mixed in the presence of serum, the number of cocci ingested varied as the temperature, from 0 to 40 C., due to the fact that there was less action on the cocci by the opsonins in the serum at low temperatures. After a long sensitization at 18 or 7 C., the action of the leukocytes was quite independent of the temperature within certain limits as 7-18, 37, 40 C.

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[21] Bull. de l'Acad. de méd., Feb. 17, 1908.

[22] Ibid., 1908.

[23] Ann. Trop. Med., 1910, 4,p. 225.

[24] Ibid., p. 510.

[25] Ztschr. Hyg. u. Infektionskr., 1900, 33, p. 289.

[26] Erkaltung und Erkaltungskrankheiten. Wien, 1907.

[27] Centralbl. f. klin. Med., 1893, 9, p. 1017.

[28] Wien. klin. Wchnschr., 1907, 20, p. 1625.

[29] Münch. med. Wchnschr., 1914, 61, p. 1721.

[30] Ann. de l'Inst. Past., 1893, 7, p. 225.

Practically all of the work on antibody production as influenced by cold has been done since 1906. One of the first series of experiments was that of Graziani,[35] who immunized rabbits with the filtrate from an 8-day broth culture of B. typhosus; three injections of increasing nonlethal doses were given under the skin of the back at intervals of two and three days. Briefly, he found that the agglutinin titer of the serum of animals kept constantly at 2-4 C. was nearly twice that of animals kept at room temperature as controls, the serum being drawn the same number of days after the last injection. In another series of experiments he kept half of a series of rabbits at 32 C. constantly during the experiment, while the other half were kept at 32 C. except for being chilled morning and evening during the period of immunization in water at 20 C. for 30 minutes, after which they were dried and put back at 32 C. The results from this series are similar to those in the first set of experiments, namely, the chilled rabbits showed on the average twice the agglutinin titer of the control animals. Graziani's method of determining the agglutinin titer was a microscopic one, the highest titer being the dilution in which there were no longer distinct clumps of 10-15 bacteria in the midst of free, slightly moving bacilli after 30 minutes.

Fukuhara,[36] in a rather incomplete experiment with 3 rabbits immunized to B. typhosus 31 days previously, found that the agglutinin and bacteriolysin content of the serum was increased in one animal cooled by dipping in cold water, this slight increase persisting for several days. The same result was obtained with a second animal kept at 10 C. for 3 hours, while keeping a third animal at 2 C. for 3 hours caused a higher agglutinin and bacteriolysin titer directly after the chilling, but the animal died on the following day and serum drawn while the animal was dying showed a decreased bacteriolysin titer while the agglutinin titer remained increased.

Trommsdorff, on the contrary, studying the agglutinin and bacteriolysin content of serum 1-8 days after the first injection of B. typhosus in guinea-pigs, found that strong cooling caused a considerable decrease in the agglutinin production. In these experiments the animals were immunized by injections of 1/10 lethal doses of typhoid bacilli, twice in the morning and twice in the afternoon on 2 successive days, and the tests for agglutination were made macroscopically. Trommsdorff showed also that guinea-pigs immunized to ox red blood cells (2 injections intraperitoneally of 5 c c washed red blood cells) showed a decreased hemolysin production after cooling during the period of immunization. In animals injected after cooling there was a decreased hemolysin production up to the 4th day after cooling. If the first injection was delayed until 5 or 6 days after the cooling there was no effect on the hemolysin production, showing that the effect of the cold had been overcome in that number of days. On a large number of animals Trommsdorff found that there was no constant effect produced on the alexin content of the blood by cooling the animals, as determined by hemolytic experiments. However, if the alexin content was decreased markedly by injection of large amounts of ox red blood cells the regeneration of alexin was very much slower in the cooled animals. He found also that there was a decreased extracellular lysis of partially hemolyzed hen's corpuscles injected into the peritoneal cavity of cooled animals.

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[31] Proc. Roy. Soc., 1905, 76, p. 706.

[32] Deut. Arch. f. klin. Med., 1908, 94, p. 335.

[33] Arch. f. Hyg., 1906, 59, p. 1-86.

[34] Centralbl. f. Bacteriol., I, O., 1908, 42, p. 632.

[35] Ibid., 1906, 42, p. 633.

[36] Arch. f. Hyg., 1908, 65, p. 275.

Finally, Lissauer[37] found that chilling rabbits, which he had previously immunized with sheep corpuscles, in water at 10 C. to the extent that the body temperature reached 30-35.2 C., caused a marked reduction (about 4 times) in the hemolytic activity of the serum drawn immediately after cooling, compared with the activity of the serum drawn just before chilling. This phenomenon persisted 2-3 hours after the cooling, although tests were not made at later periods.

I. EFFECT OF CHLLING ON HEMOLYSIN PRODUCTION FOR SHEEP CORPUSCLES

Method of Experiment.—In this series 8 rabbits were used, 4 in the chilled group and 4 as controls. Rabbits of similar weight were put opposite one another in the chilled and control groups. All 8 rabbits were immunized with washed sheep corpuscles injected intravenously in doses as follows of 50% suspension at intervals of 2 days: 1 c c, 2 c c, 3 c c, 5 c c. The rabbits in the chilled group were immersed up to their necks in ice water, of which the temperature ranged from 0-8 C., for periods of 7-10 minutes twice a day during the period of immunization. On the days of injection the rabbits were chilled for this period 20 minutes after the injection, this delay being made to allow the immediate shock of the injection to pass off before the chilling was begun, and once again some time during the day after a lapse of 6-8 hours. On the days between the injections the animals were chilled twice, once in the morning and once in the evening. Of the original 8 rabbits, 3 died, one control dying acutely from the third injection, and two chilled rabbits, one dying from snuffles contracted 3 days after chilling began, and the other dying from the effect of cold directly. The first two of these rabbits were replaced by other rabbits treated as outlined. On an average the rectal temperature dropped 8 to 9 C., in one case 12 degrees and in another case 13 degrees, and did not reach normal again for 3 or 4 hours.

Blood was drawn from the ears on the days given in Table 1. In testing the hemolytic power of the serums after inactivation at 58 C. for ½ hour, each tube contained 3 c c, 1 c c being serums diluted with normal salt solution, 1 c c being 2½% suspension of sheep corpuscles and 1 c c being 1/20 guinea-pig serum, diluted with salt solution if necessary to make 1 c c. Before using the complement it was titrated against a known amboceptor so that sufficient but not an excess would be used. All tubes were incubated for 1 hour at 37 C. The figures give the highest dilution at which complete hemolysis occurred (Table 1).

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[37] Arch. f. Hyg., 1907, 63, p. 332.

TABLE 1

HEMOLYTIC TITER OF SERUM OF DIFFERENT RABBITS

Days After First Injection	Days After Last Injection	Control Rabbits				Chilled Rabbits			
		1	2	5	7	2	4	6	8
6	...	132	133	133	80	133	133	133	133
9	3	800	800	800	800	800	800	1,333	1,000
12	6	2,000	2,000	4,000	2,000	4,000	2,000	3,000	3,000
16	10	2,000	1,333	3,000	2,000	3,000	1,333	2,000	2,000
20	14	2,000	1,333	2,000	1,000	2,000	1,000	1,000	1,000
23	17*	1,333	1,000	2,000	1,000	1,333	1,000	1,000	1,000
27	21*	400	200	1,333	500	1,000	200	dead	470
35	29*	200	150	1,000	300	500	170	dead	250

On the days marked with the asterisk the chilled rabbits were subjected to chilling 10 minutes at a time, blood being drawn immediately before chilling and immediately after (as in Lissauer's experiments) and the hemolytic titer of the serum determined. The results in Table 2 show that there was practically no difference between the 2 sets of serums.

TABLE 2

HEMOLYTIC TITER DETERMINED BEFORE AND AFTER CHILLING

Rabbit	Day After Last Injection	Titer Before Chilling	Titer After Chilling
2	17	1 : 1,333	1 : 1,333
	21	1 : 1,000	1 : 1,000
	29	500	667
4	17	1,000	1,000
	21	200	170
	29	170	170
6	17	1,000	1,000
	Dead	.....	.....
	Dead	.....	.....
8	17	1,000	1,000
	21	470	470
	29	250	250

From the data in Table 1, which gives the hemolytic titer of the serum from 8 rabbits for a period of 29 days both during and after the period of immunization, it is quite obvious that the chilling as conducted had practically no effect on the hemolysin production in Rabbits 2, 4, 6, and 8. The chilled rabbits ran as parallel to the control animals as possible, there being more individual variation between rabbits in either group than there was difference between the control and chilled groups. Trommsdorff's experience with guinea-pigs is contrary to this result, but his experiments were conducted in a different manner on a different species of animal and consequently there can be little more said in regard to the discrepancy.

Table 2 shows that the hemolysin content of the serum is not modified by chilling a rabbit in water for 10 minutes sufficiently to decrease the rectal temperature 8°–10° C. Lissauer's results, however, are entirely contrary to this result and I cannot explain or account for the discrepancies noted. If it was really the influence of cold which caused the antibody to decrease to one-fourth its concentration in the serum on account of chilling in 10° water in Lissauer's work, then it is hard to see why, if Lissauer's report is trustworthy, the rabbits in Table 2, chilled more vigorously than Lissauer's, did not show similar changes. It may be said that in my experiments the serum obtained immediately before and after chilling was titrated at the same time, the same corpuscle suspension, complement and salt solution being used, and the tubes were incubated the same length of time to the minute. Furthermore, it is hard to see how any such influence as cold could cause the removal of three-fourths of the hemolysin from the circulating blood.

II. EFFECT OF CHILLING ON THE AGGLUTININ PRODUCTION FOR BACILLUS TYPHOSUS

In this experiment 10 rabbits were used, 5 controls and 5 chilled animals, rabbits of similar weight being placed opposite one another in the 2 groups. The typhoid bacilli to be used in the immunization were grown on plain agar slants for 24 hours, the growth from each tube being washed off in 10 c c normal salt solution, and killed in a water bath at 60 C., for one-half hour. The following doses were given on the first 5 days: 0.25 c c, 0.3 c c, 0.3 c c, 0.35 c c, and 0.5 c c; 0.7 c c was given on the 7th day, 1.0 c c on the 8th, 1.3 c c on the 9th, 1.5 c c on the 10th, and 1.1 c c on the 13th. The animals of the chilled group were chilled as follows: 5 minutes on the 1st day, 6 on the 2nd, 7 on the 3rd, 7 on the 4th, 10 on the 5th, 10 on two occasions on the 6th, 10th and 7th, 12 on two occasions on the 8th, 12 on the 9th, 13 and 10 on the 10th, 12 on the 11th, 12 on the 12th, and 13 on the 13th. The method of chilling was the same as in the hemolytic experiment. The combination of cold and typhoid bacilli proved too much for 3 of the chilled rabbits, No. 4 dying on the 13th day, No. 8 on the 15th, and No. 10 on the 14th day. All the controls retained their weight and were in good health during the experiment except No. 5, which died from an accident on the 19th day.

In testing the agglutinin titer the macroscopic test tube method was used. The organisms to be agglutinated were grown on a large flat surface on plain agar in a large bottle for 24 hours, and then washed off in sufficient salt solution to make the desired turbidity. Before using they were killed by heating at 60 C. for one-half hour in the water bath. In making the tests each tube contained 1 c c of serum diluted with NaCl solution as necessary and 1 c c of typhoid suspension. All serums drawn on the same day were tested with the same typhoid suspension. All tubes were incubated 1 hour at 37 C., and the highest dilution in which agglutination was macroscopically visible was taken as the titer of the serum. Table 3 indicates the results.

TABLE 3

AGGLUTININ TITER ON DIFFERENT DAYS

Days After First Injection	Days After Last Injection	Control Rabbits					Chilled Rabbits				
		1	3	5	7	9	2	4	6	8	10
8	..	3,200	1,200	3,000	3,000	1,600	2,000	2,000	4,000	4,000	2,000
12	..	4,000	3,000	6,000	4,000	5,000	4,000	Dead	6,000	8,000	8,000
15	3	5,000	5,000	6,000	4,000	6,000	8,000	Dead	6,000	8,000	Dead
20	8	4,000	6,000	Dead	5,000	6,000	4,000	Dead	8,000	Dead	Dead
27	15	2,000	2,000	Dead	1,200	2,667	1,200	Dead	2,666	Dead	Dead

On the 15th day after the last injection the 6 rabbits alive at that time were chilled 10 minutes, blood being drawn immediately before chilling, 20 minutes after chilling, and 24 hours after chilling. Titration of the serums obtained gave the results shown in Table 4.

TABLE 4

AGGLUTININ TITER RECORDED AT DIFFERENT INTERVALS AFTER CHILLING

	1	2	3	4	7	9
Before chilling.....................	2,000	1,200	2,000	2,666	1,200	2,666
20 minutes after chilling..........	2,000	1,600	2,666	4,000	1,600	4,000
24 hours after chilling............	2,000	1,600	2,666	4,000	1,600	4,000

From the data in Table 3 it is seen that chilling to the extent described causes a moderately increased agglutinin production for B. typhosus, the average titer in the chilled animals being higher than that of the control series. This was more obvious before the 3 chilled rabbits died, as the comparison of the titers of the first 3 sets of serums shows. Although the agglutinin production was increased the chilled rabbits were more susceptible to the typhoid injections, since 3 of 5 died and the remaining 2 lost more weight and fared much less favorably than the controls. This experiment corroborates Graziani rather than Trommsdorff, whose work on guinea-pigs gave just the opposite conclusions.

The data in Table 4 show that chilling rabbits immunized 15 days previously causes an increased agglutinin content in the serum (except in Rabbit 1), this increase persisting for 24 hours. This work corroborates Fukuhara's results.

ANATOMIC CHANGES PRODUCED IN THE LUNGS BY CHILLING

Aufrecht reported some experiments which seem not to have been repeated, and as a side issue to the chief topic of this paper, the following experiments were made to see whether Aufrecht's observations could be duplicated. Briefly, he reported experiments on 3 animals with no controls which he chilled by immersing in ice water for 1, 3 and 10 minutes, respectively, on each of 3 days. He found that chilling caused coagulation of fibrin in the blood stream in the part chilled and in the lungs, the coagulation being ascribed by him to the injury to the white corpuscles by the cold. He states also that the coagulation in the lungs gave rise to hemorrhages, especially subpleural, and in the interstitial tissue. Migration of leukocytes was noticed into the adventitia of the vessels and also into the so-called thrombi. From the description the thrombi he observed might well be postmortem clots and artefacts produced by precipitation in the fixing fluid, and the special cell, "neutrophil lymphocyte," answers well the description of a polymorphonuclear leukocyte.

TABLE 5

RESULTS OF EXPERIMENTS TO DETERMINE WHETHER HEMORRHAGES AND BLOOD COAGULATION ARE DUE TO CHILLING

Rabbit	Time of Chilling	Method of Killing	Manner of Death	Macroscopic Changes	Microscopic Changes
1	1 minute on 3 successive days	Blow on neck	Severe spasms, 15 seconds	All showed numerous fresh red hemorrhages especially along margins of lungs, slightly more marked in rabbit No. 3. Hemorrhagic area varied from 1 to 8 mm. in diameter and extended into lung for 1 to 5 mm.	Fresh blood in alveoli in all sections from hemorrhagic areas. No edema. No coagulation except one small thrombus 2 mm. in diameter in No. 5. No cellular changes. No other changes
2	3 minutes on 3 successive days	Blow on neck	Severe spasms, 10 seconds		
3	10 minutes on 3 successive days	Blow cn neck	Severe spasms, 20 seconds		
4	No chilling	Blow on neck	Severe spasms, 10 seconds		
5	No chilling	Blow on neck	Severe spasms, 15 seconds		
6	No chilling	Injection of ½ gm. KCN intravenous	Extremely severe spasms	Lungs riddled with many fresh red hemorrhages 2-10 mm. in diameter; half of lung tissue hemorrhagic	
7	No chilling	Intravenous injection of chloral (1 gm.)	Quiet death; no spasms or rigor	Several small fresh bright red hemorrhages (2-5 mm.) in upper lobes	
8	10 minutes on 3 successive days	Chloralized completely per rectum; blow on neck	Severe spasms	Several small areas of fresh, bright red hemorrhages in upper lobes; more than in No. 7	
9	10 minutes on 3 successive days	Intravenous chloral injection (1.3)	Quiet death; no spasms or rigor	Only few small areas of fresh hemorrhages; less than No. 7	
10	10 minutes on 3 successive days	Intravenous injection of .66 gm. chloral	Chest opened before death; heart cut open while still beating	One small area of fresh hemorrhages (2 mm.) in each of upper lobes	No changes except small area of fresh hemorrhage noted grossly
11	Same as No. 10	Same as No. 10	Same as No. 10	One small area (2 mm.) only in right upper lobe	No changes except small area of fresh hemorrhage noted grossly
12	Same as No. 10	Same as No. 10	Animal died before chest was opened. No spasms or rigor	Several small areas of fresh hemorrhage as in Nos. 7 and 9	No changes except small area of fresh hemorrhage noted grossly

In my experiments the first 3 rabbits (1, 2, and 3) were treated as Aufrecht's rabbits (Table 5). Rabbits 6 to 9 were narcotized, before chilling, by means of chloral injection per rectum (0.7 gm.). Rabbits 10, 11, 12 were not narcotized before chilling and were treated as follows with the results shown in Table 5.

The first conclusion to be drawn is that the hemorrhages in the lungs were in all cases *fresh* hemorrhages and apparently occurred at the time of death. Furthermore, the hemorrhages occurring in the lungs of rabbits killed by means of a blow on the neck were not due to the chilling, for the controls in all cases showed as much hemorrhage as the chilled animals. Hence, the results indicate that the hemorrhages described by Aufrecht were agonal. In all cases, the amount of hemorrhage in the lung varied with the amount of spasm and rigor as the animal died. The possibility that some of the hemorrhages noticed, especially the more obscure, deeper ones, might have resulted from the struggles in the bath, led to the narcotization of Rabbits 6-9, inclusive, before chilling. However, since the findings in these rabbits are similar to those in the unnarcotized Rabbit 12 killed in a similar manner and to the findings in narcotized rabbits not subjected to cooling (No. 7), and since rabbits not narcotized and which struggled when chilled showed practically no hemorrhages when killed in a different way (Nos. 10 and 11), it is obvious that no hemorrhages resulted from the muscular efforts of the rabbits when being chilled.

Finally, the conclusion is reached that cold did not cause coagulation in the vessels of the lungs, the only thrombus found in the whole series being noticed in a control animal. Numerous structures corresponding to those described by Aufrecht as thrombi were noticed in all the lungs, but they were merely postmortem clots and artefacts produced by fixation.

SUMMARY

Chilling rabbits twice a day during the period of immunization for periods of 7-10 minutes in water at 8° C. did not cause any change in the hemolysin production for sheep corpuscles, and a single chilling 17-29 days after the period of immunization failed to change the hemolysin content of the serum.

Chilling rabbits thoroughly during the period of immunization caused a moderate increase in agglutinin production for B. typhosus. Chilling once 15 days after the period of immunization caused an increase in the agglutinin content of the serum, which increase persisted for 24 hours.

Cold baths in ice water at 0° C. for 1–10 minutes failed to cause hemorrhages or thrombosis in the lungs of rabbits.